

Chemical Exposure and Intestinal Function

by Carol M. Schiller*

The particular substances that are ingested by individuals are the consequence of their environmental, residential, and occupational exposures. The possible effects of these exposures on intestinal functions can be examined by the evaluation of *in vivo* or *in vitro* exposure followed by an *in vivo* and/or *in vitro* monitoring of effects. Several examples of the *in vivo* exposure and *in vitro* monitoring approach are presented to demonstrate the consequences of oral exposure to either a heavy metal (arsenic), or a herbicide contaminant (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) or a jet fuel propellant (hydrazine) and the subsequent measurement of either a particular metabolic pathway, or a cell-specific enzyme induction or the development of brush border enzymes are presented.

Human beings live in a very complex environment and are exposed to a multitude of natural and synthetic chemicals as illustrated in Figure 1. In addition to exposure by the skin and the respiratory system, the gastrointestinal tract provides a source of contact to a wide variety of chemicals which can be transported by a complex system (1). The kinds of substances include materials derived from ingested foods and liquids, food additives, food contaminants, drugs, particulate matter of inspired air which is swallowed, and bacterial and fungal species which proliferate within the intestines and may produce potent metabolic products. The substances ingested by an individual are the consequence of their environmental (water, air), residential (dirt, drugs) and occupational (dust, fumes) exposures.

The normal intestinal functions are summarized with examples in Table 1. An understanding of the basic principles of normal intestinal functions should permit greater appreciation for the unique roles of this organ in absorption and metabolism. In addition, an understanding of normal function may lead to better methods for detection of dysfunction and malabsorption.

In the clinical setting, intestinal dysfunction (malabsorption syndrome) is monitored by gross inspection of the feces for fat, and measurement of serum carotene, calcium, albumen and cholesterol as well as monitoring the plasma prothrombin time. Intestinal malabsorption may be reflective of properties of a particular portion of the absorptive tract and of the etiological agent involved. Some of the parameters that have been developed for clinical diagnosis are just now being assimilated into animal studies in order to develop animal models for the evaluation of possibly harmful environmental exposures of the intestinal tract.

There is a rapidly growing body of knowledge concerning the effects of ingested substances on intestinal function. The approaches utilized in the examination of altered intestinal function include: *in vivo* exposure and *in vivo* (*in situ*) evaluation; *in vivo* exposure and *in vitro* evaluation; *in vitro* exposure and *in vitro* evaluation; *in vivo* exposure and a combination of both *in vivo* and *in vitro* evaluation. Examples of these approaches are presented in Table 2.

Several examples of *in vivo* exposure and *in vitro* monitoring will be examined in depth. These indepth examples of the *in vivo* exposure and *in vitro* monitoring approach are from the research performed in my laboratory. These examples are concerned with several aspects of intestinal function both in adult and developing animals and are summarized in Table 3.

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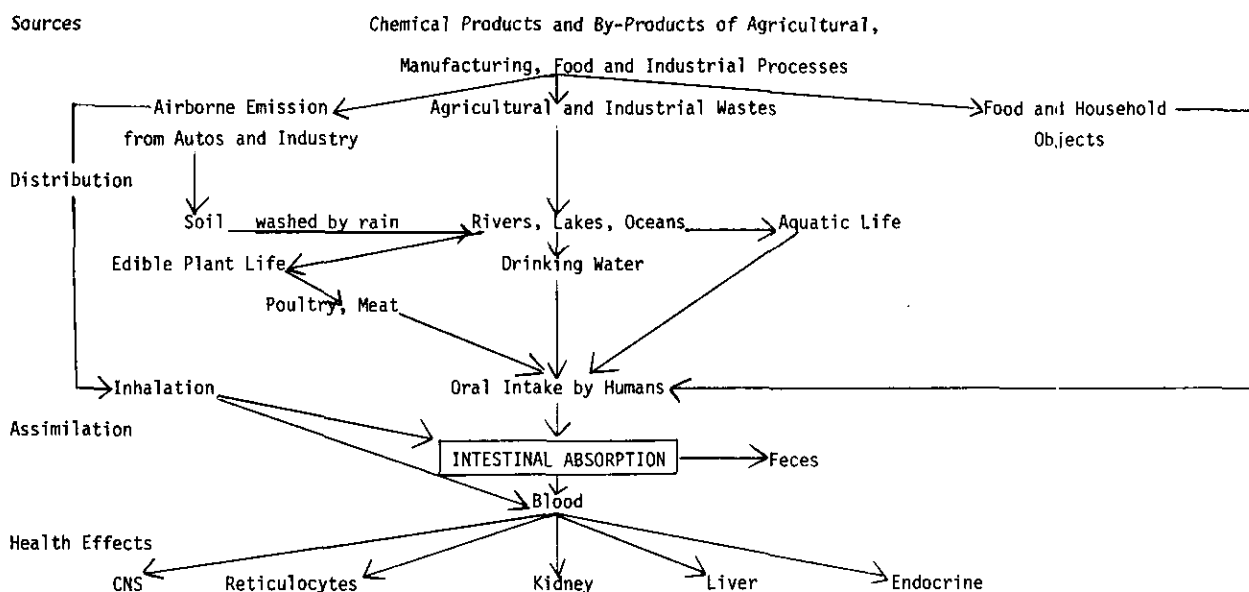


FIGURE 1. Ecodiagram of chemicals in the environment and possible effects on humans.

Alteration of a Metabolic Pathway

An example of the *in vivo/in vitro* approach which examines a metabolic pathway is provided by our work with prolonged oral arsenic exposure (9). In this study, the investigation focused on the utilization of pyruvate which is a key intermediate in intracellular metabolism. Our model system was the

adult male Charles River CD rat given access to casein-based purified diet and to deionized drinking water containing 0, 40, or 85 ppm sodium arsenate (As^{5+}) for 3 weeks. This dosage regimen was chosen on the basis of a preliminary study (10) in which 85 ppm of sodium arsenate resulted in a significant (10%) weight loss after 3 weeks of exposure, (Fig. 2). No mortality occurred among any of the treatment groups during the course of the experiment.

The results of the respiration experiments indicated depressed state 3 (ADP present) respiration and decreased respiratory control ratios for pyruvate/malate-mediated respiration but not for succinate-mediated respiration. These effects were most pronounced in animals given the highest dose of 85 ppm arsenic (As^{5+}). The ADP/O ratios were marginally depressed for both substrates at the highest arsenic dose level. These results indicated that mitochondrial oxidation of pyruvate/malate differed

Table 1. Normal intestinal functions.

Digestion: hydrolysis of foodstuffs
Secretion: mucopolysaccharides, water, electrolytes, hormones
Absorption: nutrients, ions, vitamins, water
Metabolism: synthesis, degradation
Detoxification: conjugation, hydroxylation, hydrolysis
Immune response: local antibodies, IgA
Structural barrier: selective penetration
Elimination: motility
Colonization: enteric metabolism

Table 2. Evaluation of altered intestinal function.

Method of exposure/ method of monitoring	Function studied	Environmental agent	Reference
<i>In vivo/in situ</i>	Absorption of Ca	Cadmium	(2)
<i>(in vivo)</i>	Motility	Teratogens	(3)
<i>In vivo/in vivo</i>	Secretion of water	Diquat	(4)
	Absorption of nutrients	Parathion	(5)
<i>In vitro/in vitro</i>	Transport of nutrients	Pesticides	(6)
<i>In vivo/combination</i>	Absorption of Ca and Zn	PCB (Aroclor 1242)	(7)
	growth ultrastructure		
	Metabolism	Cadmium	(8)

Table 3. Applications of *in vivo* exposure and *in vitro* monitoring.

Exposure/monitoring	Example
Prolonged oral exposure of adult male rats/metabolic pathways	Arsenic/intermediary metabolism of pyruvate
Single intubation of adult female rats/cell-specific enzyme induction	TCDD/UDP-glucuronyltransferase levels in isolated tip and crypt cells
Single intubation of date-bred female hamsters/enzyme development	Hydrazine/ontogeny of brush border enzymes

from that of succinate. We then utilized a radiochemical assay technique (11, 12) to investigate the mitochondrial enzyme pyruvate dehydrogenase, which exists both in an active (unphosphorylated) form and in an inactive (phosphorylated) form. Exposure of animals to arsenic for 3 weeks resulted in a decrease in both the basal (inactive) and total (active) pyruvate dehydrogenase activities as shown in Table

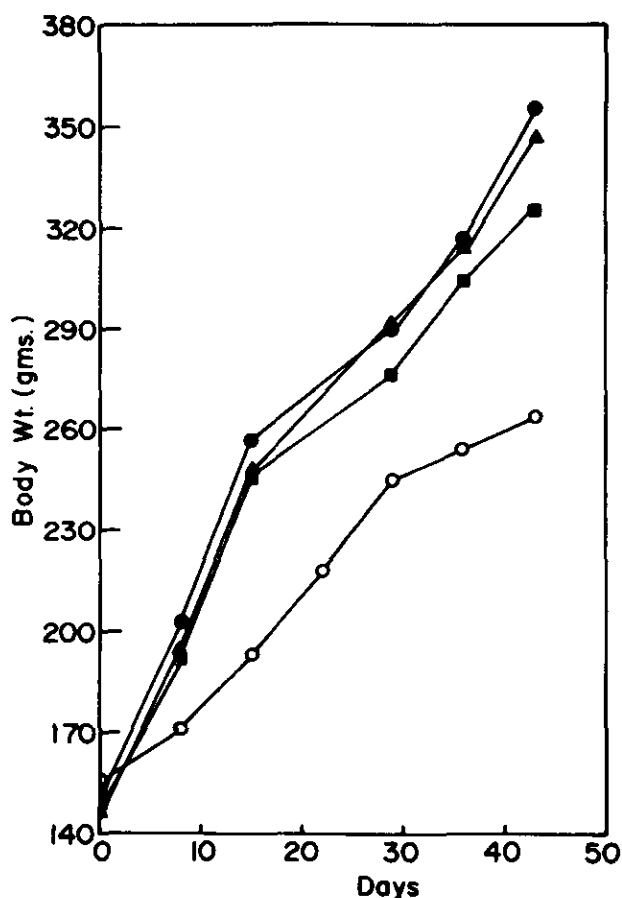


FIGURE 2. Growth curves for rats given a purified diet and arsenate (As^{3+}); (●) control; (Δ) 20 mg $As/l.$ drinking water; (■) 40 mg/l.; (○) 85 mg/l. Significant ($p < 0.01$) depression of growth occurred at only the 85 ppm dose level (10).

4. This study suggested that *in vivo* exposure to arsenic decreases both the basal and total levels of pyruvate dehydrogenase activity in liver and intestine. In addition, the relative ease of activation appeared to increase after arsenic exposure. Because of the central role of pyruvate dehydrogenase in the mitochondrial utilization of pyruvate, the observed diminished pyruvate dehydrogenase activity after exposure to arsenic may decrease the flow of acetyl-CoA into the tricarboxylic acid cycle as well as decrease the amount of fatty acid synthesis (Fig. 3). The selective effects of arsenic on mitochondria were also reflected in the decreased rate of oxidation of pyruvate by mitochondria isolated after treatment and in the abnormal ultrastructural architecture in these same animals (10).

Cell-Specific Enzyme Induction

A second example of the *in vivo* exposure and *in vitro* monitoring approach involved the study of the effects of an environmental agent, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), on cell-specific enzyme induction (13). TCDD is an extremely toxic contaminant produced during the commercial synthesis of the herbicide 2,4,5-trichlorophenoxyacetic acid. A striking feature of the well documented toxicity of TCDD is the minute

Table 4. Inhibition of rat liver and intestinal pyruvate dehydrogenase activity after 3 weeks exposure to arsenate.^a

As^{3+} , ppm	Pyruvate dehydrogenase, % Inhibition			
	Liver		Intestine	
	Basal	Total	Basal	Total
40	23	15	29	27
85	48	28	37	32

^aBasal and total refer to pyruvate dehydrogenase activity before and after Mg-activation *in vitro*. Details of the experimental design, conditions for activation, and assay have been reported previously (9).

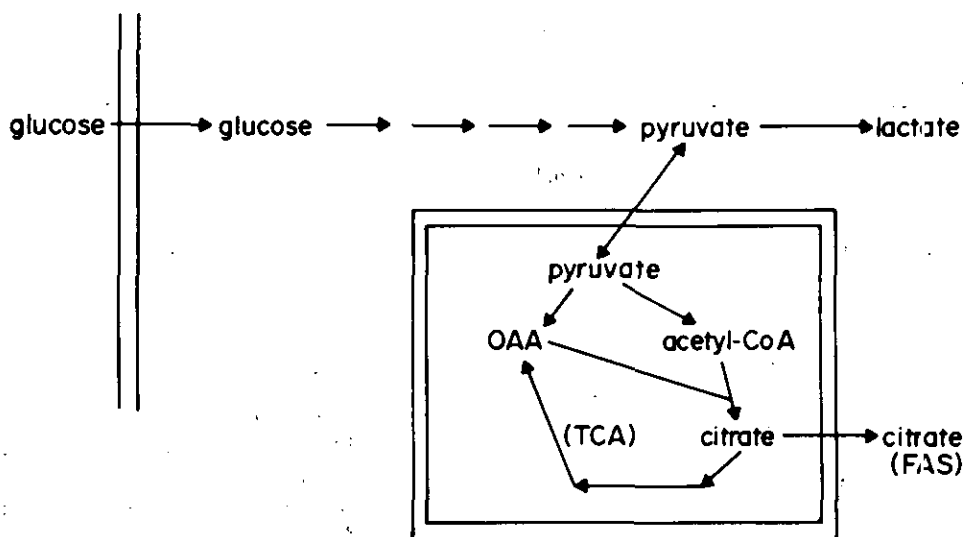


FIGURE 3. Schematic illustration of the flow of glucose metabolites into the mitochondria. TCA, tricarboxylic acid cycle; FAS, fatty acid synthesis (9).

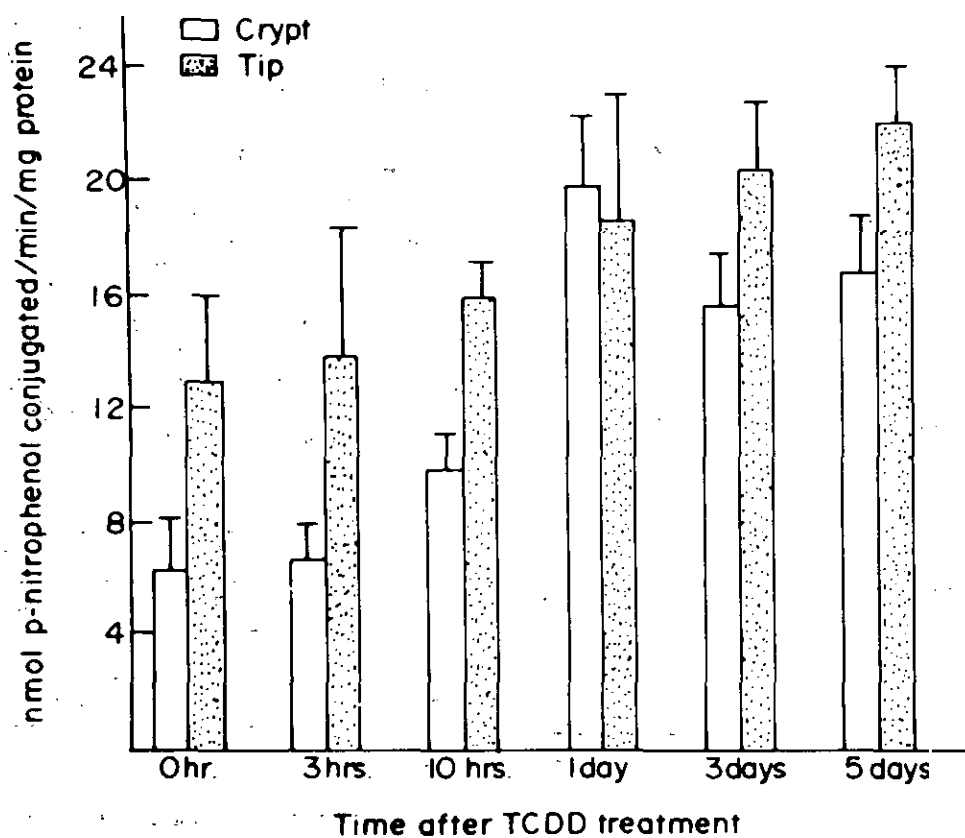


FIGURE 4. Time-course effects of TCDD on UDP-glucuronyltransferase activity in intestinal crypt and tip cells; $N = 3$. Each value represents the mean ± 1 S.D. (13).

quantity of TCDD required to produce pathogenic effects. Although the biochemical lesions underlying the observed toxicity of TCDD are not entirely understood, the activities of the UDP-glucuronyltransferase and aryl hydrocarbon hydroxylase are induced with nonlethal doses in rodent intestines (14-16). In our study, the TCDD-mediated induction of UDP-glucuronyltransferase was monitored in cells isolated from the tip and crypt regions of the intestines. The sensitivity of the isolated tip and crypt cells to this TCDD-mediated induction of UDP-glucuronyltransferase was determined by measuring time-dependent alterations in the level of enzyme induction and the concentrations of ^{14}C -activity in each of these cell types. The differential vibration method was used for the sequential removal of the intestinal cells (17). The effectiveness of this process was verified by morphological examination of the cells and by measurement of levels of appropriate marker enzymes (13).

Adult female rats were fasted for 16 hr and then fed ad libitum after receiving a single oral dose at $8\text{ }\mu\text{g/kg}$ of ^{14}C -TCDD. [The LD_{50} value for a single oral dose of TCDD is approximately $100\text{ }\mu\text{g/kg}$ (18).] *p*-Nitrophenol glucuronidation was measured 0, 3, and 10 hr and 1, 3, and 5 days after treatment. Basal activities of the crypt and tip cells were approximately 6.5 and 13 nmole *p*-nitrophenol conjugated/min-mg protein, respectively (Fig. 4). UDP-glucuronyltransferase activities were approximately the same as control values 3 hr following TCDD treatment. However, after 10 hr, tip cell activity increased by 20% as compared to a 50% elevation of activity in crypt cells. The differential induction between the intestinal cell types became more pronounced by 1 day after TCDD administration, i.e., 200% increase in crypt cell activity and 50% increase in tip cell activity (Fig. 4).

The greater sensitivity of the crypt cells to the TCDD-mediated effects of the intestine suggested a more general sensitivity of undifferentiated cells to inductive agents. Evidence for this greater sensitivity of the crypt cells to the actions of TCDD is provided by the fact that UDP-glucuronyltransferase induction in crypt cells occurs prior to the elevation of the tip cell enzyme activity (Fig. 4) and by the negative correlation between the level of UDP-glucuronyltransferase induction and the measured levels of ^{14}C -activity in these same cell preparations (Fig. 5). Estimates of the time required for the transit of the crypt cells to the tip of the villus, where they are sloughed off, are usually between 48 and 72 hr (19, 20). During this period, the crypt cells lose their capacity to proliferate and become adapted to per-

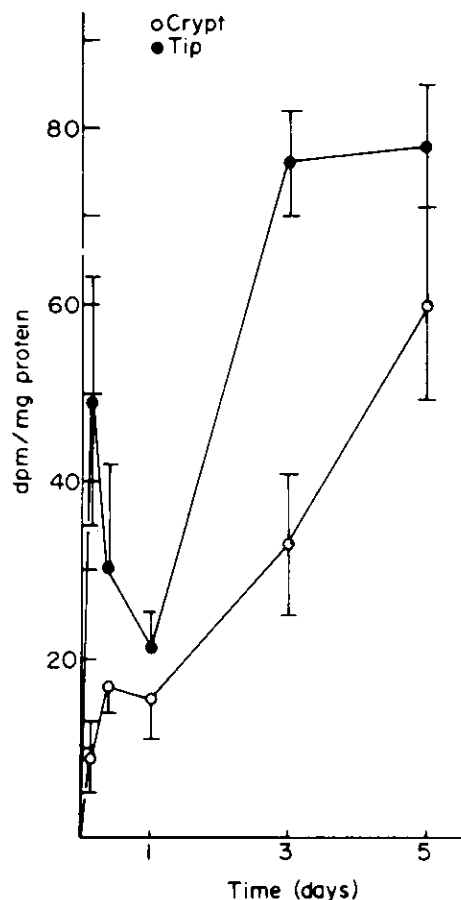


FIGURE 5. Time course of ^{14}C -TCDD incorporation and retention in intestinal crypt and tip cells. Each animal was given $0.73\text{ }\mu\text{Ci}$ of ^{14}C -TCDD (148 mCi/mmol) as described in the text (13).

form absorptive functions. The levels of UDP-glucuronyltransferase normally increase 2-fold in this transition period (Fig. 4). The time required for the appearance of marked UDP-glucuronyltransferase induction in the tip cells correlates well with the crypt to tip migration time of 2-3 days (Fig. 5). Our observation that the levels of ^{14}C -activity in both cell types were highest 3-5 days after treatment (Fig. 5) is consistent with the results of an earlier pharmacokinetic study (21).

The elevated levels of ^{14}C -activity observed in the isolated cells may, in part, be a reflection of the enterohepatic circulation of TCDD or a more polar metabolite. No attempts have been made at this time to determine the cellular distribution of the ^{14}C -activity in the isolated tip and crypt cells or the chemical nature of the ^{14}C -labeled material. The role of enterohepatic circulation in the exposure of the

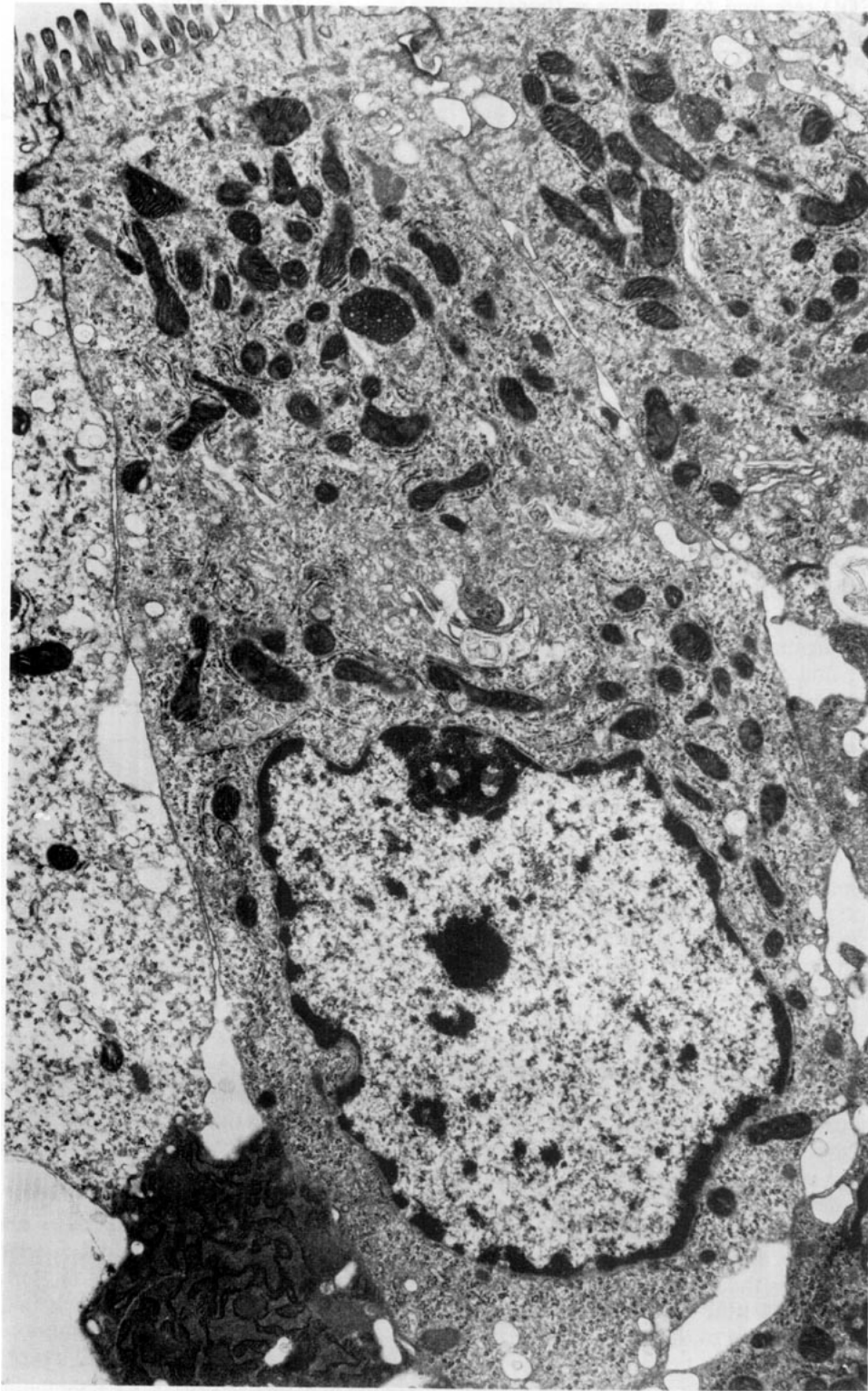


FIGURE 6. Transmission electron photomicrograph of isolated intestinal cells prepared by vibration of rat duodenum. Method of preparation has been described in detail (13, 17). 11,628 \times .

intestine to metabolic products of ingested substances is receiving increasing attention. Of particular interest is the enterohepatic circulation of metabolites of hydrazine and its methylated derivatives.

Altered Intestinal Development

The last approach I will mention involves an investigation of altered intestinal function resulting from prenatal exposure to hydrazine and its methylated derivative, 1,2-dimethylhydrazine, which is a colon-specific carcinogen. The effects of prenatal exposure on normal intestinal enzyme development were examined (22) by monitoring three brush border enzymes, lactase, sucrase, and alkaline phosphatase, each of which has a unique metabolic function and, a different developmental pattern. After prenatal exposure to 1,2-dimethylhydrazine, the postnatal level of sucrase activity and all levels of alkaline phosphatase activity were elevated. In contrast, exposure to hydrazine diminished neonatal lactase, elevated postnatal and young adult sucrase, and elevated the neonatal and postnatal activities of alkaline phosphatase. Early changes resulting from an in utero exposure to these compounds may be an indication of an insult which is later expressed in pathological alteration to intestinal tissues such as tumor formation. Whereas altered enzyme activities are being utilized currently as indices of toxicity in general toxicology studies (9, 13, 23, 24), the altera-

tions we observed permit monitoring of altered enzyme activities in a specific organ after prenatal exposure to a carcinogen specific for that organ.

A model for studying prenatal exposure and intestinal function of offspring has been reported recently (3). In that instance fetal rats were exposed to a teratogen, either actinomycin-D, or hydroxyurea, or methotrexate, and then evaluated by the postnatal function tests of gastrointestinal transit time, rectal emptying, and gastric emptying (3).

Another study described the fetal and postnatal tissue distribution of several ^{14}C -labeled chlorinated biphenyl after prenatal exposure (25). Chlorinated biphenyls that did not accumulate significantly in adipose tissue did accumulate dramatically in fetal intestine. Radioactivity was cleared rapidly from the intestine after parturition. When 1-CB was administered at the 15th day of gestation, intestinal accumulation still did not occur until the 19th day of gestation, corresponding with the developmental onset of hepatic glucuronyltransferase. Approximately 10% of the fetal intestinal radioactivity following 1-CB treatment was in the parent compound, 5% was in 4-hydroxy-4-chlorobiphenyl, and 50% was in the glucuronide of 4-hydroxy-4-chlorobiphenyl. It is proposed that the chlorinated biphenyls are hydroxylated in the maternal compartment, and that the hydroxylated biphenyl metabolite is transferred across the placenta and conjugated by UDP-glucuronyltransferase in the fetal liver. The glucuronide that reaches the intestine cannot be ex-

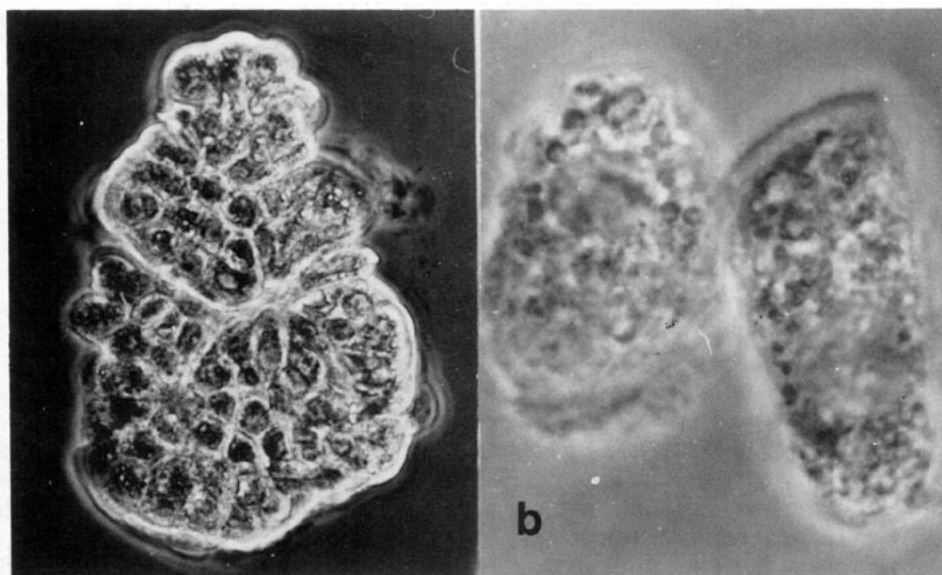


FIGURE 7. Phase-contrast photomicrographs of isolated intestinal cells prepared by vibration: (a) clump of cells, 200 \times ; (b) isolated cells, 300 \times .

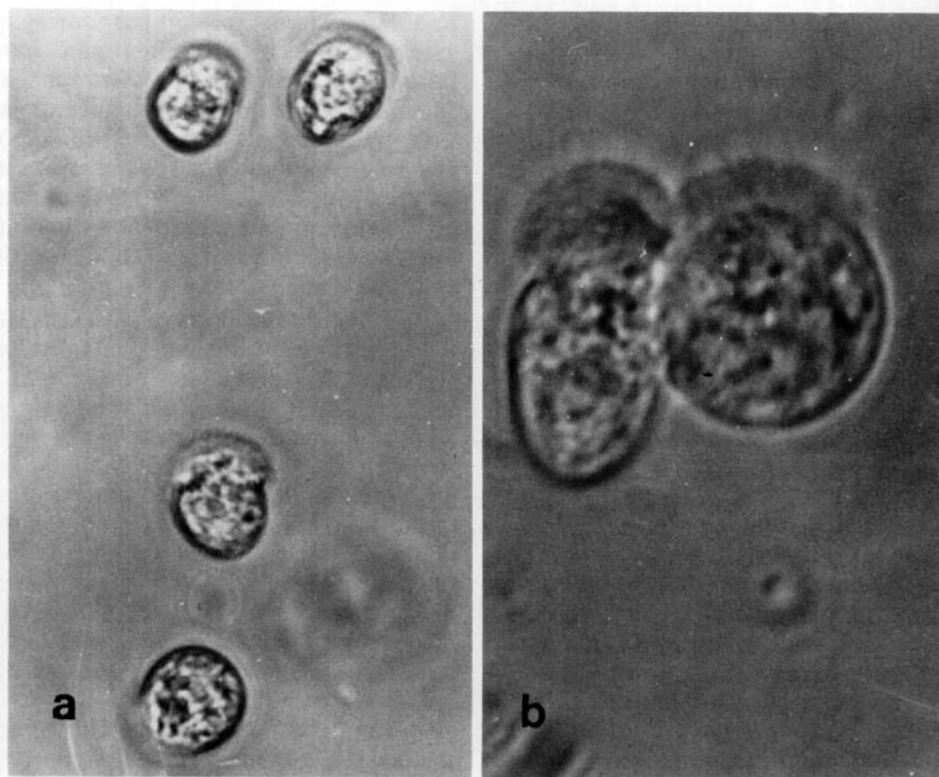


FIGURE 8. Phase-contrast photomicrograph of isolated intestinal cells prepared by protease/EDTA. The everted duodenal segments were filled to slight distention and the villous absorptive cells were harvested by incubation in a protease (10 U/ml)/EDTA (1 mM) medium. The medium was Hanks' balanced salt solution (Ca, Mg, and glucose free) which contained bovine serum albumin (0.5% w/v) and Tris-HCl (20 mM), pH 7.4. The initial incubation with protease/EDTA in medium for 10 min was followed by incubation with medium only for 15 min. The cells released in the second incubation were washed twice in medium by centrifugating at 150g for 1 min at 4°C and resuspending in fresh medium: (a) monodisperse suspension of isolated cells, 250 \times ; (b) 300 \times .

creted, but can be hydrolyzed by β -glucuronidase. The aglycone can then be absorbed into the fetal circulation and reconstituted in the fetal liver. We have monitored several intestinal enzyme development patterns in these offspring but found no conveniently altered indicator enzyme. This study suggests, however, a model for the study of fetal intestinal exposure as well as a mechanism for the metabolism of this class of chemicals by enzymes in the several compartments involved.

At this point it may be appropriate to discuss the use of isolated cells for *in vitro* studies. We have observed that the tip cells (absorptive cells) prepared by the differential vibration technique maintain the characteristic columnar morphology of the absorptive intestinal cell (Fig. 6) of intact tissue. However, cell counts are difficult because of the aggregation of these cells either as a consequence of the mucus present or because the cells were isolated as clumps

initially (Fig. 7). For studies that examine the *in vitro* uptake and metabolism of substances, we have developed another technique which involves a protease/EDTA treatment. The cells obtained by this enzyme/chelation method are monodispersed suspensions of absorptive cells (Fig. 8) and retain normal cellular membranous and subcellular organization as observed by transmission electron microscopy (Fig. 9). These cells have normal enzyme complements and are active metabolically.

Whereas these studies of the evaluation of *in vivo* exposure by *in vitro* monitoring may represent interesting and informative examples of the effects of environmental agents on intestinal functions, they provide, at best, only a beginning of future research in this area. There is a recent review which summarizes the information available on the gastrointestinal responses to drugs and dietary test substances (1) as well as a review of the environmental



FIGURE 9. Transmission electron photomicrograph of isolated intestinal cells prepared by protease/EDTA. 11,628 \times .

factors involved in the development of injury and disease to the gastrointestinal tract (26). Together, these serve as a starting point for gastrointestinal toxicology.

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